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Diaphragmed fenestrae in the glomerular endothelium versus nondiaphragmed fenestrae in the hepatic endothelium

To the Editor: In a recent issue of *Kidney International*, Ballermann [1] discussed the development of diaphragmed fenestrae in glomerular endothelial cells. Major similarities exist between the hepatic and the glomerular endothelium regarding the process of fenestrae formation; intriguingly, an analogous controversy about the origin of fenestrae exists in both the hepatology and nephrology fields [2].

Hepatic endothelial fenestrae are dynamic structures that act as a sieving barrier to control the extensive exchange of material between the blood and the liver parenchyma. These membrane-bound pores lack a diaphragm and a basal lamina is also absent (Fig. 1 A and B, arrows), in contrast to fenestrae described in the kidney, pancreas, and brain. Their biological relevance in various diseases has been widely acknowledged [3].

Evidence that VEGF-induced diaphragmed fenestrae are derived from fused caveolae has recently been accumulating. It has been postulated that a similar mechanism is used in the formation of hepatic fenestrae. Conversely, actin disruption in hepatic endothelial cells produces an increase in the number of fenestrae by using a distinct structure that appears to serve as a fenestrae-forming center (Fig. 1C) [4]. Furthermore, it is important to emphasize the contradictions in the data reported about the origin of fenestrae in kidney, pancreas, and in the liver. For example, some data either clearly or indirectly indicate a possible relationship between caveolae, vesiculovacuolar organelles, and (diaphragmed) fenestrae; others suggest the opposite (reviewed in [2]). Therefore, until this controversy is settled, no firm conclusions can be drawn, and stating that fenestrae, whether diaphragmed or not, correspond to fused and interconnected caveolae should be viewed with caution and remains as an emerging research topic.

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Reply from the Authors

Braet and Soon question the conclusions we have drawn in a recent review [1] about the relationship between caveolae and fenestrae in glomerular endothelium. They suggest that we were too hasty in stating that fenestrae do not represent fused caveolae.

There is little doubt about the dynamic nature of fenestrae. Work with liver sinusoidal endothelial cells is particularly interesting because these cells retain very nice sieve plate structures in culture which can change rapidly by alterations in actin microfilament dynamics [2]. Also, VEGF-A can induce endothelial fenestration. Conversely, interference with VEGF-A function in renal glomeruli, for instance, in preeclampsia [3] or with podocyte VEGF-A haploinsufficiency [4] leads to an absence of glomerular fenestrae. Finally, VEGF-A seems to induce caveolin-1–containing vesicles to fuse and to form transendothelial cell channels [5, 6]. Such circumstantial evidence has suggested that fenestrae represent fused caveolae.

However, in caveolin-1–deficient mice known to lack caveolae altogether [7], we found glomerular endothelium with fenestrae that were normal in size and density, even though no caveolin-1 and no caveolae were observed at all [8]. In normal rat glomeruli examined for caveolin-1 localization by immunogold electron microscopy, fenestrated portions of glomerular

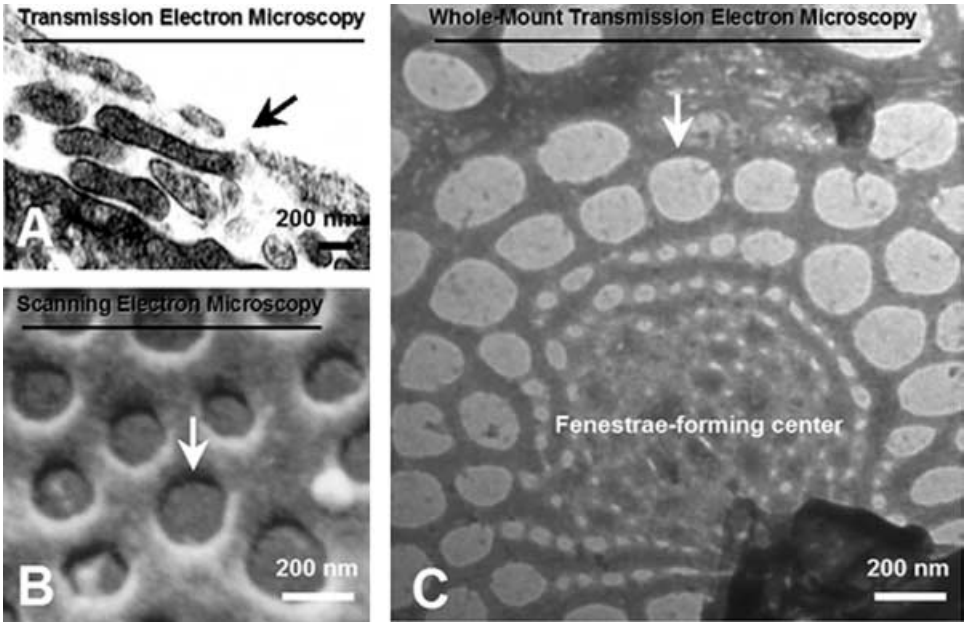

Fig. 1. High magnification electron micrographs of rat hepatic endothelial fenestrae. (*A*) Transmission electron microscopy image of sectioned hepatic endothelial cells *in situ*, showing the fenestrated, processes (arrow) and the histologic relationship with the numerous microvillous processes of the liver parenchymal cells situated in the space of Disse. (*B*) Scanning electron micrograph of the fenestrated cytoplasm of hepatic endothelial cells *in vitro*. Notice the fenestrae (arrow) on the cell surface. (*C*) Whole-mount transmission electron micrograph of the fenestrated cytoplasm (arrow) of a hepatic endothelial cell cultured on transmission electron microscopy grids, disclosing the process of fenestrae formation. Note the area of intermediated density from which rows of fenestrae with increasing diameter are radiating into the surrounding cytoplasm. Scale bars, 200 mm.

endothelium were completely devoid of caveolin-1, similar to the report by Esser et al [9] for the choroid plexus endothelium. Now, our experiments leave open the possibility that fenestrae in other vascular beds could represent fused caveolae, and that caveolar precursor vesicles lacking caveolin-1 could fuse to form fenestrae. But if caveolae are defined as specialized plasma membrane invaginations stabilized by caveolins, then our findings in caveolin-deficient mice seem to prove that fenestrae in glomerular endothelium cannot represent fused caveolae.

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Immunosuppressive and calcimimetic drug-drug interactions

To the Editor: Drug-drug interactions (DDIs) are an important cause of adverse drug reactions. It has been estimated that approximately 5% of prescribing errors [1] or of adverse drug reactions [2] are caused by DDIs in